

Rec'd PTO 16 JAN 2002  
09/830669  
#14

205907US-0 PCT

IN THE UNITED STATES PATENT & TRADEMARK OFFICE

IN RE APPLICATION OF :

PHILIPPE MARLIERE ET AL : EXAMINER:

SERIAL NO: 09/830,669 :

FILED: APRIL 30, 2001 : GROUP ART UNIT:

FOR: METHOD FOR PRODUCING IN :  
VIVO PROTEINS CHEMICALLY  
DIVERSIFIED BY  
INCORPORATING NON-STANDARD  
AMINO ACIDS

PRELIMINARY AMENDMENT

ASSISTANT COMMISSIONER FOR PATENTS  
WASHINGTON, D.C. 20231

SIR:

Responsive to the Official Correspondence dated November 16, 2001, Applicants submit herewith a Sequence Listing, a corresponding computer-readable Sequence Listing, and an amendment to the specification. Prior to examination on the merits, please amend the above-identified application as follows.

IN THE SPECIFICATION

Page 20, please replace the paragraph comprising lines 34 and 35 as follows:

--Oligodeoxynucleotide 1 (SEQ ID NO:1):

5'pTGGATAAAATGGCGCTGGCACCGGTACATGCATTCTCCAGTTCTATGT--

Page 21, please replace the paragraph comprising lines 28-31 as follows:

--Oligodeoxynucleotide 3 SEQ ID NO: 3): 5'GGTGTGATCATGATGGTC

Oligodeoxynucleotide 4 SEQ ID NO: 4): 5'CCTGCAAGATGGATTCCC

Oligodeoxynucleotide 5 SEQ ID NO: 5): 5'CGCGCCGCATTATTGTTTC

Oligodeoxynucleotide 6 SEQ ID NO: 6): 5'GTCTGGACCGGTGGCGACA--

Page 22, please replace the paragraph comprising lines 35-36 as follows:

--Oligodeoxynucleotide 2 (SEQ ID NO: 2):

5'pTGGATAAAATGGCGCTGGCACCGATACATGCATTCCAGTTCTATGT--

Page 25, please replace the paragraph comprising lines 5-8 as follows:

--Oligodeoxynucleotide 7 (SEQ ID NO: 7):

5'GGGAATTCGGTGTGAAATTGCCGCAGAACG

Oligodeoxynucleotide 8 (SEQ ID NO: 8):

5'GGCAAGCTTCCAGTATTCACGGGGAGTTATGC--

Page 41 (Abstract), after the last line, beginning on the next page, please insert the Sequence Listing attached hereto.

#### REMARKS

Claims 1-51 are active in the present application.

Applicants have now submitted a Sequence Listing and a corresponding computer-readable Sequence Listing. Contents of the paper copy of the Sequence Listing and the computer-readable Sequence Listing are identical. Support for all the sequences listed in the Sequence Listing can be found in the present application. No new matter is introduced by the submission of the Sequence Listing and the computer-readable Sequence Listing.

Applicants submit that this application is in condition for allowance. Early notice to this effect is earnestly solicited.

Respectfully submitted,

OBLON, SPIVAK, McCLELLAND,  
MAIER & NEUSTADT, P.C.



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**Marked-Up Copy**  
Serial No: 09/830,669  
Amendment Filed: Herewith

IN THE SPECIFICATION

Page 20, please replace the paragraph comprising lines 34 and 35 as follows:

--Oligodeoxynucleotide 1[:] (SEQ ID NO:1):

5'pTGGATAAAATGGCGCTGGCACCGTACATGCATTCTCCAGTTCTATGT--

Page 21, please replace the paragraph comprising lines 28-31 as follows:

--Oligodeoxynucleotide 3[:] SEQ ID NO: 3): 5'GGTGTGATCATGATGGTC

Oligodeoxynucleotide 4[:] SEQ ID NO: 4): 5'CCTGCAAGATGGATTCCC

Oligodeoxynucleotide 5[:] SEQ ID NO: 5): 5'CGCGCCGCATTATTGTTTC

Oligodeoxynucleotide 6[:] SEQ ID NO: 6): 5'GTCTGGACCGGTGGCGACA--

Page 22, please replace the paragraph comprising lines 35-36 as follows:

--Oligodeoxynucleotide 2[:] (SEQ ID NO: 2):

5'pTGGATAAAATGGCGCTGGCACCGATAACATGCATTCTCCAGTTCTATGT--

Page 25, please replace the paragraph comprising lines 5-8 as follows:

--Oligodeoxynucleotide 7[:] (SEQ ID NO: 7):

5'GGGAATTCGGTGTGAAATTGCCGCAGAACG

Oligodeoxynucleotide 8[:] (SEQ ID NO: 8):

5'GGCAAGCTTCCAGTATTACGGGGAGTTATGC--

09/830669

JC08 Rec'd PCT/PTO 30 APR 2001

205907US0 PCT

IN THE UNITED STATES PATENT & TRADEMARK OFFICE

IN RE APPLICATION OF : :

PHILLIPPE MARLIERE ET AL : ATTN: NEW APPLICATION DIVISION

SERIAL NO: NEW US PCT APPLN. :  
(Based on PCT/FR99/02628)

FILED: HEREWITH : :

FOR: PROCESS FOR PRODUCING  
CHEMICALLY DIVERSIFIED  
PROTEINS, *IN VIVO*, BY  
INCORPORATING  
UNCONVENTIONAL AMINO  
ACIDS

#3/a  
3/a

7-23-01

PRELIMINARY AMENDMENT

ASSISTANT COMMISSIONER FOR PATENTS  
WASHINGTON, D.C. 20231

SIR:

Prior to examination on the merits, please amend the above-identified application as follows:

IN THE CLAIMS

Please amend the claims as shown in the marked-up copy to read as follows:

--3. (Amended) Method according to claim 1, characterized in that step c) for  
culturing said cells comprises a series of said cells in a culture medium containing the amino  
acid encoded by said target codon, each of said cultures of the series being prepared as far as  
obtaining the stationary growth phase and followed by washing of the cells obtained, the  
number of cultures of the series being sufficient to allow the selection of mutations which  
increase the suppression of said missense mutation of said mutated gene.

4. (Amended) Method according to claim 1, characterized in that the missense mutation is chosen from missense mutations which spontaneously reverse only at very low frequency, of the order of one organism from at least  $10^{15}$ .

5. (Amended) Method according to claim 1, characterized in that the missense mutation transforms a target codon of a gene encoding a protein required for the growth of said cell, into a codon which, in comparison with the target codon, exhibits a change of at least two bases, preferably three bases.

*A<sup>1</sup>  
and*  
6. (Amended) Method according to claim 1, characterized in that the target codon encodes an amino acid which has a small steric volume.

7. (Amended) Method according to claim 1, characterized in that the target codon encodes an amphiphilic amino acid.

8. (Amended) Method according to claim 1, characterized in that the target codon encodes an amino acid which has a steric volume smaller than or substantially equal to the steric volume of the amino acid encoded by the missense mutation.

9. (Amended) Method according to claim 5, characterized in that the target codon encodes cysteine.

10. (Amended) Method according to claim 5, characterized in that the amino acid encoded by the missense mutation is valine or isoleucine.

11. (Amended) Method according to claim 1, characterized in that step a) for transforming said cells is carried out using a vector comprising a sequence of said gene encoding a protein required for the growth of said cells, including said missense mutation.

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12. (Amended) Method for selecting cells capable of producing a protein the amino acid sequence of which comprises at least one unconventional amino acid, characterized in that it comprises steps a), where appropriate b), and c) of a method according to claim 1, and

*a2 gene'*  
selecting the cells capable of growing in step c).

15. (Amended) Method for selecting cells according to claim 13, characterized in

*AB* that the aminoacyl-tRNA synthetase which recognizes the amino acid encoded by said missense mutation of said selected cells is capable of charging onto one of its associated tRNAs an unconventional amino acid or an amino acid other than said amino acid encoded by said missense mutation.

*A4* 18. (Amended) Cell obtained using a method according to claim 1.

*AB* 20. (Amended) Cell according to claim 18, characterized in that it is a prokaryotic or eukaryotic cell.

*A4* 22. (Amended) Cell according to claim 18, characterized in that it is chosen from the following cells deposited at the CNCM (Collection Nationale de Culture de Microorganismes [National Collection of Microorganism Cultures], Paris, France):

- a) *E. coli* strain deposited at the CNCM under the No. I-2025 on May 25, 1998,
- b) *E. coli* strain deposited at the CNCM under the No. I-2026 on May 25, 1998,
- c) *E. coli* strain deposited at the CNCM under the No. I-2027 on May 25, 1998,
- d) *E. coli* strain deposited at the CNCM under the No. I-2339 on-October 26, 1999,
- e) *E. coli* strain deposited at the CNCM under the No. I-2340 on October 26, 1999,

and

- f) *E. coli* strain deposited at the CNCM under the No. I-2341 on October 26, 1999.

23. (Amended) Use of a method according to claim 1 for producing protein the amino acid sequence of which comprises at least one unconventional amino acid.

24. (Amended) Use of a cell according to claim 18 for producing protein the amino acid sequence of which comprises at least one unconventional amino acid.

25. (Amended) Process for producing a protein the amino acid sequence of which comprises at least one unconventional amino acid, characterized in that it comprises the following steps:

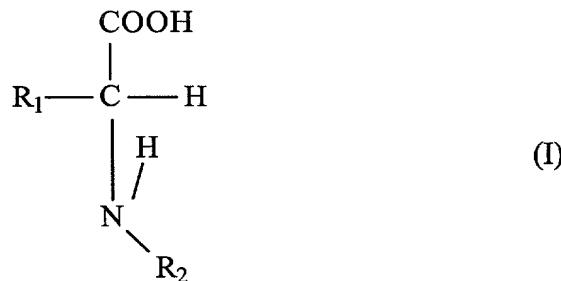
- a) where appropriate, selecting a cell by a method according to claim 13;
- b) culturing said cell selected in step a) in a culture medium and under culture conditions which allow the growth of said cell; and
- c) isolating said protein comprising at least one unconventional amino acid from the culture supernatant and/or from the cell pellet obtained in step b).

29. (Amended) Process according to claim 25, characterized in that said cell is auxotrophic for the amino acid encoded by said target codon.

30. (Amended) Process according to claim 25, characterized in that said cell comprises a homologous or heterologous gene of interest the coding sequence of which includes at least one target codon.

32. (Amended) Process according to claim 30, characterized in that the biological activity of the protein encoded by said gene of interest is at least partially conserved after the incorporation of said unconventional amino acid at the level of the target codon of said gene of interest.

33. (Amended) Process according to claim 25, characterized in that the unconventional amino acid is chosen from unconventional amino acids of formula I of configuration L



in which:

$\text{R}_1$  or  $\text{R}_2$  represents radicals containing a functional group capable of reacting selectively.

35. (Amended) Process according to claim 25, for protein functionalization.

36. (Amended) Protein purification process, characterized in that it comprises the following steps:

a) incorporating into the amino acid sequence of said protein an unconventional amino acid containing a functional group capable of reacting selectively, using a process according to claim 25;

b) bringing the solution containing the protein obtained in step a) into contact with a support comprising a compound capable of reacting specifically with said functional group and of attaching specifically said protein; and

c) isolating said protein attached to the support.

37. (Amended) Process for attaching a protein to a chemical or biochemical compound, characterized in that it comprises the following steps:

a) incorporating into the amino acid sequence of said protein, by a process according to claim 25, an unconventional amino acid containing a functional group capable of reacting selectively;

*Gene*  
b) bringing the protein obtained in step a) into contact with said chemical or biochemical compound comprising a group capable of reacting specifically with said functional group in a medium allowing the reaction.

*A10*  
41. (Amended) Process according to claim 39, characterized in that the chemical or biochemical compound is chosen from compounds capable of modifying the biological activity of the attached protein.

42. (Amended) Process according to claim 39, characterized in that the chemical or biochemical compound is chosen from compounds the biological activity of which can be modified by the attached protein.

43. (Amended) Process according to claim 39, characterized in that the chemical or biochemical compound is chosen from compounds comprising a protein, a polynucleotide, a fatty acid, a sugar or a natural or synthetic polymer.

44. (Amended) Protein obtained using a process according to claim 25.

46. (Amended) Protein complex obtained using a process according to claim 39.

47. (Amended) Use of a protein according to claim 44, as a diagnostic reagent.

*A11*  
48. (Amended) Diagnostic process, characterized in that it uses a protein according to claim 44.

*Add claims*  
*A12*  
49. (Amended) Diagnostic pack, characterized in that it contains a protein according to claim 44.

50. (Amended) Use of a protein according to claim 44 for preparing a pharmaceutical or cosmetic composition.

51. (Amended) Pharmaceutical or cosmetic composition comprising a protein according to claim 44.--

49. Diagnostic pack, characterized in that it contains a protein according to claim 44 or 45, or a protein complex according to claim 46.

50. Use of a protein according to claim 44 or 45, of a protein complex according to claim 46 or of a cell according to one of claims 18 to 22 for preparing a pharmaceutical or cosmetic composition.

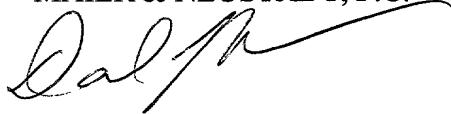
51. Pharmaceutical or cosmetic composition comprising a protein according to claim 44 or 45, a protein complex according to claim 46 or a cell according to one of claims 18 to 22.

REMARKS

Claims 1-51 are active in the present application. The claims are amended to remove multiple dependencies. No new matter is added. An action on the merits and allowance of the claims is solicited.

Respectfully submitted,

OBLOON, SPIVAK, McCLELLAND,  
MAIER & NEUSTADT, P.C.



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**Marked-Up Copy**  
Serial No: \_\_\_\_\_  
Amendment Filed on:  
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IN THE CLAIMS

Please amend the claims as follows:

--3. (Amended) Method according to [either of claims 1 and 2] claim 1, characterized in that step c) for culturing said cells comprises a series of said cells in a culture medium containing the amino acid encoded by said target codon, each of said cultures of the series being prepared as far as obtaining the stationary growth phase and followed by washing of the cells obtained, the number of cultures of the series being sufficient to allow the selection of mutations which increase the suppression of said missense mutation of said mutated gene.

4. (Amended) Method according to [one of claims 1 to 3] claim 1, characterized in that the missense mutation is chosen from missense mutations which spontaneously reverse only at very low frequency, of the order of one organism from at least  $10^{15}$ .

5. (Amended) Method according to [one of claims 1 to 4] claim 1, characterized in that the missense mutation transforms a target codon of a gene encoding a protein required for the growth of said cell, into a codon which, in comparison with the target codon, exhibits a change of at least two bases, preferably three bases.

6. (Amended) Method according to [one of claims 1 to 5] claim 1, characterized in that the target codon encodes an amino acid which has a small steric volume.

7. (Amended) Method according to [one of claims 1 to 6], claim 1, characterized in

that the target codon encodes an amphiphilic amino acid.

8. (Amended) Method according to [one of claims 1 to 7] claim 1, characterized in that the target codon encodes an amino acid which has a steric volume smaller than or substantially equal to the steric volume of the amino acid encoded by the missense mutation.

9. (Amended) Method according to [one of claims 5 to 8] claim 5, characterized in that the target codon encodes cysteine.

10. (Amended) Method according to [one of claims 5 to 9] claim 5, characterized in that the amino acid encoded by the missense mutation is valine or isoleucine.

11. (Amended) Method according to [one of claims 1 to 10] claim 1, characterized in that step a) for transforming said cells is carried out using a vector comprising a sequence of said gene encoding a protein required for the growth of said cells, including said missense mutation.

13. (Amended) Method for selecting cells capable of producing a protein the amino acid sequence of which comprises at least one unconventional amino acid, characterized in that it comprises steps a), where appropriate b), and c) of a method according to [one of claims 1 to 12] claim 1, and selecting the cells capable of growing in step c).

15. (Amended) Method for selecting cells according to [either of claims 13 and 14] claim 13, characterized in that the aminoacyl-tRNA synthetase which recognizes the amino acid encoded by said missense mutation of said selected cells is capable of charging onto one of its associated tRNAs an unconventional amino acid or an amino acid other than said amino acid encoded by said missense mutation.

18. (Amended) Cell obtained using a method according to [one of claims 1 to 17] claim 1.

20. (Amended) Cell according to claim 18 [or 19], characterized in that it is a

prokaryotic or eukaryotic cell.

22. (Amended) Cell according to [one of claims 18 to 21] claim 18, characterized in that it is chosen from the following cells deposited at the CNCM (Collection Nationale de Culture de Microorganismes [National Collection of Microorganism Cultures], Paris, France):

- a) *E. coli* strain deposited at the CNCM under the No. I-2025 on May 25, 1998,
- b) *E. coli* strain deposited at the CNCM under the No.I-2026 on May 25, 1998,
- c) *E. coli* strain deposited at the CNCM under the No. I-2027 on May 25, 1998,
- d) *E. coli* strain deposited at the CNCM under the No. I-2339 on-October 26, 1999,
- e) *E. coli* strain deposited at the CNCM under the No. I-2340 on October 26, 1999,

and

- f) *E. coli* strain deposited at the CNCM under the No. I-2341 on October 26, 1999.

23. (Amended) Use of a method according to [one of claims 1 to 17] claim 1 for producing protein the amino acid sequence of which comprises at least one unconventional amino acid.

24. (Amended) Use of a cell according to [one of claims 18 to 22] claim 18 for producing protein the amino acid sequence of which comprises at least one unconventional amino acid.

25. (Amended) Process for producing a protein the amino acid sequence of which comprises at least one unconventional amino acid, characterized in that it comprises the following steps:

- a) where appropriate, selecting a cell by a method according to [one of claims 13 to 17] claim 13;
- b) culturing said cell selected in step a) [or a cell according to one of claims 18 to 22]

in a culture medium and under culture conditions which allow the growth of said cell; and

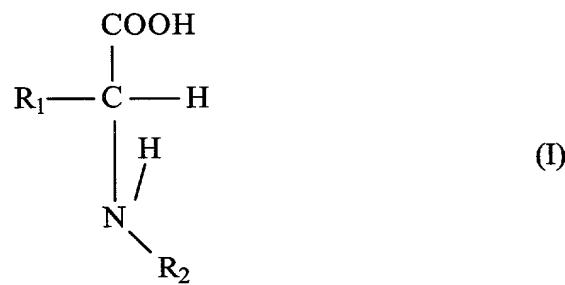
c) isolating said protein comprising at least one unconventional amino acid from the culture supernatant and/or from the cell pellet obtained in step b).

29. (Amended) Process according to [one of claims 25 to 28] claim 25, characterized in that said cell is auxotrophic for the amino acid encoded by said target codon.

30. (Amended) Process according to [one of claims 25 to 29] claim 25, characterized in that said cell comprises a homologous or heterologous gene of interest the coding sequence of which includes at least one target codon.

32. (Amended) Process according to claim 30 [or 31], characterized in that the biological activity of the protein encoded by said gene of interest is at least partially conserved after the incorporation of said unconventional amino acid at the level of the target codon of said gene of interest.

33. (Amended) Process according to [one of claims 25 to 32] claim 25, characterized in that the unconventional amino acid is chosen from unconventional amino acids of formula I of configuration L



in which:

R<sub>1</sub> or R<sub>2</sub> represents radicals containing a functional group capable of reacting

selectively.

35. (Amended) Process according to [one of claims 25 to 34] claim 25, for protein functionalization.

36. (Amended) Protein purification process, characterized in that it comprises the following steps:

- a) incorporating into the amino acid sequence of said protein an unconventional amino acid containing a functional group capable of reacting selectively, using a process according to [one of claims 25 to 35] claim 25;
- b) bringing the solution containing the protein obtained in step a) into contact with a support comprising a compound capable of reacting specifically with said functional group and of attaching specifically said protein; and
- c) isolating said protein attached to the support.

37. (Amended) Process for attaching a protein to a chemical or biochemical compound, characterized in that it comprises the following steps:

- a) incorporating into the amino acid sequence of said protein, by a process according to [one of claims 25 to 35] claim 25, an unconventional amino acid containing a functional group capable of reacting selectively;
- b) bringing the protein obtained in step a) into contact with said chemical or biochemical compound comprising a group capable of reacting specifically with said functional group in a medium allowing the reaction.

41. (Amended) Process according to claim 39 [or 40], characterized in that the chemical or biochemical compound is chosen from compounds capable of modifying the biological activity of the attached protein.

42. (Amended) Process according to claim 39 [or 40], characterized in that the

chemical or biochemical compound is chosen from compounds the biological activity of which can be modified by the attached protein.

43. (Amended) Process according to [one of claims 39 to 42] claim 39, characterized in that the chemical or biochemical compound is chosen from compounds comprising a protein, a polynucleotide, a fatty acid, a sugar or a natural or synthetic polymer.

44. (Amended) Protein obtained using a process according to [one of claims 25 to 36] claim 25.

46. (Amended) Protein complex obtained using a process according to [one of claims 39 to 43] claim 39.

47. (Amended) Use of a protein according to claim 44 [or 45, or of a protein complex according to claim 46], as a diagnostic reagent.

48. (Amended) Diagnostic process, characterized in that it uses a protein according to claim 44 [or 45, or a protein complex according to claim 46].

49. (Amended) Diagnostic pack, characterized in that it contains a protein according to claim 44 [or 45, or a protein complex according to claim 46].

50. (Amended) Use of a protein according to claim 44 [or 45, of a protein complex according to claim 46 or of a cell according to one of claims 18 to 22] for preparing a pharmaceutical or cosmetic composition.

51. (Amended) Pharmaceutical or cosmetic composition comprising a protein according to claim 44 [or 45, a protein complex according to claim 46 or a cell according to one of claims 18 to 22].--